primary and tertiary structures around the Cu_A site employing amino acid sequence analysis and structural modeling, and found that the presence/absence of an *inserted structural element* close to the Cu_A site may be related to the diversity of the redox potential.

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20P9

Interfacial water provides the pathway for proton transport along membranes

Denis Knyazev, Andreas Springer, Peter Pohl
Institut für Biophysik, Johannes Kepler Universität Linz, Gruberstrasse 40, 4020 Linz, Austria
E-mail: peter.pohl@jku.at

Proton diffusion along membrane surfaces is thought to be crucial for many cellular processes such as energy transduction. It is commonly regarded as a succession of jumps between membrane-anchored proton binding sites. Our experiments provide evidence for an alternative model. We released protons at the interface, and monitored their arrival at distant sites by fluorescence measurements. The kinetics of the arrival was probed as a function of distance (i) for membranes of various compositions [1] and (ii) for the decane/water interface [2]. We found that long-range proton diffusion along the interface required neither the presence of ionizable groups [1] nor of lipids [2]. Salt removal altered the diffusion constant but did not inhibit long range lateral proton migration. Surface to bulk transfer was delayed by an energy barrier, which according to measurements at various temperatures amounted to at least 8.7 kT [2]. The observation of a large isotope effect supported the conclusion that interfacial water provided the pathway for rapid lateral proton migration.

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20P10

Microanalysis of the quinone reducing site Qi from the cytochrome *b6f* complex

Audrey Lebas[1], Francesca Zito[1], Fabrice Rappaport[2], Frauke Baymann[3], Daniel Picot[1]
[1]*UMR 7099 Institut de Biologie Physico-Chimique, 13,rue Pierre et Marie Curie, 75005 Paris, France*
[2]*UMR 7141 Institut de Biologie Physico-Chimique 13, rue Pierre et Marie Curie, 75005 Paris, France*
[3]*BIP, CNRS, UPR9036, IFR88, 31, chemin Joseph Aiguier, 13009 Marseille, France*
E-mail: Daniel.Picot@ibpc.fr

Most electron transfer chains share a complex from the Rieske/cytochrome *b* family. A common mechanism based on the Q-cycle is usually widely accepted. Yet, the quinone reducing site Q_i of the complex harbors an additional heme, called ci, in cyanobacteria and chloroplast, heliobacteria and low GC gram positive bacteria. The configuration of that haem suggests a possible coordination with a substrate, an unusual feature for supposed electron transfer site. Despite the accumulation of data, the mode of action of the action of the Q_i site remains to be elucidated. We propose here that the use of anomalous scattering can be used to overcome the limiting resolution of the available structures in order to gain further insights on the heme states and to correlate spectroscopic and crystallographic data.

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20P11

Heterologously expressed Aio: A system to study biogenesis and structure/function relationships of the Rieske superfamily

R. van Lis, W. Nitschke, T.P. Warelow, L. Capowiez, J.M. Santini, B. Schoepp-Cothenet
Laboratoire de Bioénergétique et Ingénierie des Protéines UMR 7281 CNRS/AMU, FR3479, F-13402 Marseille Cedex 20, France
Institute of Structural and Molecular Biology, UCL, Gower Street London WC1E 6BT, UK
E-mail: schoepp@imm.cnrs.fr

Structural similarities between the small AioB subunit of arsenite oxidase Aio, harboring a [2Fe-2S] cluster, and PetA, the Rieske-subunit of Rieske/cyt *b* complex, show that AioB is a member of the Rieske protein superfamily. AioB and PetA indeed are so closely related that the conspicuous absence of the canonical disulfide bridge in several AioB proteins, presenting yet unchanged spectral and redox properties, was surprising [1]. This disulfide bridge is considered to be essential for Rieske cluster redox and spectral properties [2,3]. AioB furthermore distinguishes itself from PetA through the apparent fate of its leader sequence. Despite a similar predicted N-terminal Twin-arginine translocation (Tat) signal sequence, PetA invariably is membrane-anchored *via* its uncleaved Tat signal peptide, whereas Aio was either found in the periplasm or associated with the cytoplasmic membrane, depending on the species [4,5]. Heterologous expression of Aio from *Ralstonia* sp. S22 and *Rhizobium* sp. NT-26 in *Escherichia coli* allowed us to address both a) the nature of Aio's membrane-association by biochemistry and b) the influence of the disulfide bridge in this enzyme by EPR. The results with the *Ralstonia* sp. S22 enzyme suggest that the Tat signal sequence is sufficient to attach the enzyme to the membrane. The study of a Cys106Ala mutant, devoid of the first Cys involved in the disulfide bridge formation, confirmed that this bridge has no significant influence on properties of the Rieske protein from Aio. Our study furthermore revealed an oxidation-induced EPR spectral conversion of AioB centre. We propose an interaction between the [3Fe-4S]- and the [2Fe-2S]-center to be responsible of this conversion.

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